BIO-INK FOR 3D PRINTING

RELATED APPLICATION

[0001] This application claims priority from Australian Provisional Patent Application No. 2018902674 filed 24 Jul. 2018 titled "Bio-ink for 3D printing", the entire contents of which are hereby incorporated by cross reference.

TECHNICAL FIELD

[0002] This technology relates broadly to bio-inks for 3D bioprinting. In particular, this technology relates to a 3D printed hydrogel formed from a polymer cross-linked using a cross-linking agent, processes for preparing the hydrogel, and uses thereof.

BACKGROUND

[0003] Cells exist three-dimensionally within an extracellular matrix (ECM) in vivo. Interactions between the cells and the ECM play an important role in controlling the biological characteristics and behaviours of cells. In cancer biology, three-dimensional (3D) in vitro cell culture assays are used to culture cells in an environment that closely mimics the native environment of the cells. Such assays maintain the physiological cell-cell and cell-matrix interactions which are absent in two-dimensional monolayer cultures.

[0004] 3D bioprinting uses 3D printing technology to combine cells and other biomaterials to prepare synthetic structures that mimic the ECM. Commonly, a 3D bioprinter utilises either a drop-on-demand or an extrusion printing technology to print 3D in vitro assays. Although drop-on-demand bioprinting has been reported to maintain high cellular viability upon printing, this technique remains less commonly utilised compared to its extrusion counterpart due to the low viscosity and low cell density of current 3D bio-inks.

[0005] Hydrogels, both synthetic and natural, have been used extensively as ECM mimics. In particular, synthetic hydrogels may be highly reproducible and offer excellent control over the physical and cell responsive properties, which are highly desirable of an ECM mimic for in vitro studies. Current approaches for the manual creation of 3D in vitro assays predominantly utilise Matrigel® or collagen as the ECM mimic. Other materials that have been used extensively are hyaluronic acid, chitosan and alginate. As they are sourced from natural products, natural hydrogels are typically biocompatible and, in some cases, carry the necessary biomolecules for cell-matrix interactions. However, natural hydrogels have limitations as ECM mimics as they may be susceptible to batch-to-batch variability, lack physical and biochemical modularity, and/or be difficult to handle.

[0006] Various synthetic hydrogels have been prepared in an attempt to overcome the various limitations of natural hydrogels. In particular, covalently cross-linked synthetic hydrogels can provide a more robust and mechanically accurate system than natural hydrogels. However, many of these synthetic hydrogels lack biochemical characteristics that promote cell-matrix interactions. Further, to apply covalently cross-linked synthetic hydrogels to the 3D bioprinting of cells, the materials should be biocompatible in order to allow cells to be printed in situ within the hydrogel. For example, while poly(acrylamide) is a biocompatible synthetic hydrogel used in cell biology (Caliari and Burdick, 2016), the corresponding hydrogel precursor is not biocompatible, thus making it unsuitable for the 3D bioprinting of cells.

[0007] Current approaches to 3D bioprinting involve UV-initiated radical cross-linking reactions that allows rapid hydrogel formation (Murphy and Atala, 2014; Donderwinkel et al, 2017: Jungst et al, 2016 Lowe et al, 2014). For example, inkjet printing of photocross-linkable acrylated PEG and peptide was found to be suitable for generating a 3D hydrogel construct for the encapsulation of mesenchymal stem cells (Gao et al, 2017; Gao et al, 2015). However, while the potential use of this approach for 3D bioprinting of living cells has been shown, the use of UV irradiation to initiate photo-cross-linking can cause damage to the DNA in living organisms, while the generated free radicals may also damage sensitive cells.

[0008] Advances in 3D bioprinting have been significant in recent years and have the potential to alleviate the limitations of current approaches to 3D in vitro cell culture assays. However, although the benefits of 3D printed cell cultures are well established, utilisation of 3D printed in vitro assays in cell biology is still constrained by their complexity, labour intensive and low-throughput nature. Accordingly, there is a need for alternative ECM mimics for use in 3D bioprinting.

SUMMARY

[0009] The present inventors have developed bio-inks suitable for 3D printing that are biocompatible and can form hydrogels rapidly via a substantially non-toxic chemical pathway.

[0010] In a first aspect, the present technology provides a 3D printed hydrogel formed from a maleimide containing polymer cross-linked using a bis-thiol containing cross-linking agent having at least two thiol functional groups.

[0011] The bis-thiol containing cross-linking agent may comprise more than two thiol functional groups.

[0012] In an embodiment, formation of the hydrogel occurs upon combining a solution comprising the maleimide containing polymer (polymer bio-ink) and a solution comprising the bis-thiol containing cross-linking agent (activator) using a 3D printer.

[0013] In an embodiment, the 3D printed hydrogel is formed within about 30 minutes or less, or 10 minutes or less, or 1 minute or less, or 30 seconds or less, or 10 seconds or less, or 1 second or less, from the printing of the polymer bio-ink and the activator.

[0014] In an embodiment, the polymer bio-ink is biocompatible with cells.

[0015] In an embodiment, the activator is biocompatible with cells.

[0016] In an embodiment, the polymer bio-ink and the activator when combined during 3D printing form a hydrogel that is biocompatible with cells.

[0017] In an embodiment, the maleimide containing polymer is selected from maleimide containing polysaccharides, such as polymers containing fructose, sucrose or glucose monomers; synthetic polymers, such as poly(ethylene glycol) (PEG) maleimide, poly(hydroxyethyl methacrylate (PHEMA) maleimide, poly(ε-caprolactone) (PCL) maleimide, poly(vinyl alcohol) (PVA) maleimide, poly(vinylpyrrolidone) (PVP) maleimide, poly(N-isopropylacrylamide) (NIPAAM) maleimide, poly(propylene fumarate) (PPF) maleimide, poly(ethylene imine) (PEI) maleimide, poly(3-